Mast Cells: Molecular And Cell Biology

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Abstract

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INTRODUCTION
Mast cells are important effector cells providing granule and membrane mediators as well as cytokines in allergic and inflammatory diseases. The study of surface molecules such as immunoglobulin receptors and adhesion molecules have greatly expanded our understanding of mast cell physiology. Activatory and inhibitory receptors have been defined with critical roles in maintaining cell functional activity. An active role for mast cells in antigen presentation to T cells has recently been shown, and direct interaction between mast cells and B cells providing signals for specific IgE production has been demonstrated. Functional receptors other than the high affinity IgE (FceRI) have been implicated in the anaphylactic response of IgE-deficient mice, suggesting that IgG receptors present in mast cells can be involved in immediate hypersensitivity reactions. A protective role for mast cells has recently been established through the release of TNF alpha in acute septic peritonitis.

Although metachromatic mast cells are easily recognized in peripheral tissues, little is known about the phenotype of mast cell precursors, their fate from the bone marrow to the tissues, the migration and homing processes and the factors and adhesion molecules that affect those processes. This review will describe recent advances in mouse and human mast cell biology and ontogeny.

MAST CELL ONTOGENY AND DEVELOPMENT

1. ROLE OF C-KIT PROTOONCOGENE AND STEM CELL FACTOR (SCF)
Mutant mice of the W/W and Sl/Sl phenotypes lack mature mast cells and exhibit hypoplastic anemia, hypopigmentation and sterility. The W locus encodes the c-kit protooncogene, a member of the immunoglobulin superfamily within the receptor tyrosine kinase family. The Sl locus codes for the ligand of c-kit, also called stem cell factor (SCF) or kit-ligand (KL). Kitamura and coworkers have shown that bone marrow transplantation from +/+ normal mice or Sl/Sld mutant mice cured the mast cell deficiency in the W/Wv deficient mice, reflecting a hematopoietic defect in the mast cell lineage. The deficiency in the Sl mutants could not be cured with bone marrow transplantation, indicating that the defect was in the non-hematopoietic microenvironment. W and Sl mutations profoundly affect the mast cell lineage, indicating that c-kit and Stem Cell Factor (SCF) are crucial to the development of mast cells. The proliferation, differentiation, chemotaxis and secretory functions of mouse, rat and human mast cells are all affected by the interactions between c-kit and SCF.

Elegant in vivo studies by Galli and coworkers of non-human primates such as baboons and cynomolgus monkeys treated subcutaneously with recombinant human SCF (rhSCF) for 21 or 28 days have shown increased mast cell numbers in multiple organs. Mast cell hyperplasia varied considerably according to the different anatomical sites with a predominant pattern at spleen, bone marrow and lymph nodes. At 100 g/kg/day mastocytosis occurred at the site of injection, bone marrow, mesenteric lymph nodes, liver and spleen, a pattern similar to that seen in some patients with systemic mastocytosis. When rhSCF was discontinued and the monkeys maintained for 15 days without it, the
numbers of mast cells declined and were undistinguishable from the control non-treated monkeys. Systemic expansion and contraction of the mast cell population occurred without any observed side effects. In vitro data suggests that SCF promotes mast cell survival by suppressing apoptosis (13).

Point mutations of the cytoplasmic tyrosine domain of the c-kit protooncogene receptor have shown a factor independent development of rat and mouse mast cells. The mouse P815 mast cell line and the rat RBL-2H3 line have an Asparagine substituted by a Tyrosine at amino acids 814 and 817 respectively (16,17). Both cell lines have been shown to have activated c-kit kinase and do not need SCF for growth and survival.

2. HUMAN MASTOCYTOSIS AND MAST CELL PRECURSORS

Increased numbers of mast cells are seen in human mastocytosis, which can be localized to the skin (as in solitary mastocytomas or urticaria pigmentosa lesions) or can involve various organs (bone marrow, bone, liver, spleen, lymph nodes) as seen in systemic mastocytosis (13). The phenotype of the hyperplastic mast cells is no different than that of the normal mast cells at the site of localization. The cause of human mastocytosis is still unclear but c-kit and SCF have been both implicated in the pathogenesis. Earlier studies on cutaneous mastocytosis (urticaria pigmentosa) have shown an excess of soluble SCF in lesional tissue sections by immunohistochemistry, in which the cellular source was not identified (18). A post-translational defect with local increase in SCF in these patients was thought to be responsible for the reactive mast cell hyperplasia. In patients with systemic mastocytosis and associated myelodysplastic syndrome a point mutation has been identified in the catalytic domain of c-kit that causes an Asp816-Val substitution (19), which is also found in the HMC1 human mast cell leukemia line (20). This mutation is analogous to the ones found in the rat mast cell line RBL-2H3 and the mouse mast cell line P815 both of which induce ligand-independent mast cell growth and have tumorogenic potential. Recently the same Asp816-Val mutation has been found in patients with only urticaria pigmentosa and in patients with aggressive mastocytosis (type III) without hematologic malignancy (21). A new mutation has been established in the same tyrosine domain of c-kit with the substitution Asp820-Gly (22) in a patient with aggressive mastocytosis. Since the reported mutations occur in both benign and malignant forms of the disease other associated factors or mutations might be important for the development of aggressive or malignant mastocytosis.

Mast cells, although having their origin in the bone marrow and expressing hematopoietic surface markers, do not circulate in the blood as recognizable, mature cells (3). In humans, circulating precursor mast cells express the CD34 epitope and can be differentiated in vitro into metachromatic, tryptase positive mast cells under the influence of SCF (4). Cord blood as well as fetal liver mononuclear cells have also been shown to be a source of mast cell progenitors (5-9). However, the identity of the circulating progenitors has not been elucidated, and little is known about the differentiation pathways that lead to mature, tissue mast cells.

Phenotypic identification of immature mast cells has been hampered by the low frequency of those circulating cells and the lack of specific mast cell surface markers at the early stages of development. Systemic mastocytosis has provided an in vivo model for the study of mast cell early forms since increased number of circulating mast cell precursors is expected as a reflection of the increased number of mast cells in the tissues. The phenotype of circulating early mast cell forms is described in a patient with type III systemic mastocytosis in which no hematological malignancy was apparent, nor were mature mast cells present in the peripheral blood. An agranular non metachromatic mononuclear cell was identified at low frequency (1% to 3%) in the peripheral blood that expressed at least 3 granule proteins (tryptase, Carboxipeptidase A (CPA) and Cathepsin G) the surface marker c-kit and lacked chymase (22). The same mononuclear agranular tryptase, c-kit, CPA and Cathepsin G positive cell was present in the spleen and lymph nodes in association to hyperplastic mature mast cells. This circulating cell that lacks metachromasia but expresses tryptase and c-kit is a likely candidate for previously unrecognized mast cell circulating progenitors (Figure 1). Surface immunohistochemical staining for SCF was shown in these mononuclear mast cell progenitors, suggesting the possibility of an autocrine regulation of their proliferation and differentiation (Figure 2). A committed precursor for the mast cell lineage in murine fetal blood has also been identified. The murine promastocyte expresses low levels of the membrane bound T cell antigen Thy1, high levels of c-kit, has mRNA for the mast cell granule proteases MMCP2, MMCP4 and CPA, lacks FceRI alpha chain and is weakly metachromatic (23). Its phenotype shares similarities with the precursor identified in the aggressive mastocytosis patient described above (27) indicating that non-
metachromatic mast cell precursors travel in the blood stream that can now be recognized with appropriate phenotypic characterization.

3. MAST CELL HETEROGENEITY

Morphology and metachromasia have been the two fundamental criteria for the recognition of tissue mast cells. The description of mast cells as mononuclear metachromatic cells needs to be amplified to include cells with multilobulated nuclei. Human mast cells can have segmented nuclei analogous to mouse mast cells which can possess multilobular nuclei at various tissue localizations. Those multilobulated mast cells express the normal protease phenotype as well as c-kit and have an electron microscopy structure similar to mononuclear mast cells at the same tissue localization. Metachromasia is found in mature mast cell but precursor, immature, degranulated and atypical mast cells may demonstrate limited or no metachromasia while they express granule and membrane specific proteins.

Biochemical evidence of mast cell heterogeneity in humans is based on the presence of proteases, which are the bulk of the protein content of the granules. Although the initial studies by Schwartz at al. indicated the presence of only two distinct types of mast cells, it is clear that there are at least three types of mature mast cells based on immunochemical studies. Mast cells containing only tryptase and named MCt predominate in the intestinal mucosal and alveoli and are scroll-rich; mast cells containing tryptase, chymase and carboxypeptidase and named MCtc predominate in skin and intestinal submucosa and have lattice-rich and amorphous granules; and the less frequent MCC only containing chymase is found in the intestinal submucosa and nasal mucosa. Intestinal MCt are decreased in AIDS and severe immunodeficiencies while MCtc are unchanged in those diseases. This indicates a CD4+ T cell dependency for the MCt. MCt from different tissues may represent a heterogeneous population itself, since different several tryptase transcripts have been isolated. Five highly homologous tryptase cDNAs (92% overall identity) have been cloned from human lung mast cells (alpha and beta forms) and from human skin mast cells (I,II and III forms with a 98% identity with the beta form) with different N-linked glycosylation patterns. Although both specific alpha and beta tryptase mRNAs are transcribed in tissue-derived mast cells, beta transcripts seem to predominate in skin and lung, and HMC-1 (human leukemic mast cell line) cells which have no detectable alpha transcripts. Expression of tryptases seems therefore tissue regulated and suggests a more complex heterogeneity pattern than reported. Tryptase purified from lung and other human sources has been shown to cleave bronchodilator peptides (vasoactive intestinal polypeptide VIP, peptide histidine methionine and vasodilator calcitonin gene-related peptide), has mitogenic activity for fibroblasts and epithelial cells, activates collagenase from synovial cells, stimulates the release of IL-8 and upregulates the expression of ICAM-1 on epithelial cells.

Murine mast cell heterogeneity is now being shown at the level of cytokine transcription and translation. Immunohistochemical and in situ RNA hybridization studies have looked at the distribution of IL-4, IL-5 and IL-6 proteins and mRNA in human skin, nasal and bronchial tissues. IL-4 is found preferentially in the MCtc subset, including skin with little IL-4 present in MCt (15%). IL-5 and IL-6 are restricted to the MCt subset. These findings are consistent with a different profile of cytokine generation and release depending on mast cell localization and suggest specific biologic functions for the different mast cells subsets.

4. CYTOKINES IN MAST CELL DEVELOPMENT

Originally described by Kitamura, the reconstitution of mast cell-deficient mice with bone marrow cells or with bone marrow derived mast cells cultured with IL3 indicated that under the influence of tissue factors a common precursor could give rise to all types of mature differentiated mast cells. Numerous cytokines have been identified that influence the in vitro expression of proteases in mouse mast cells. A combination of IL-9 and SCF (c-kit ligand) will induce expression of all known mouse proteases as in the population of mature mast cells found in liver and spleen. Human CD34 positive cells develop into an almost pure mast cell population in the presence of a combination of SCF and IL-6. These cultured human mast cells express functional high affinity IgE receptors (FceRI) and are predominantly of the MCt type containing only tryptase, with a minority (30%) being of the MCtc type containing tryptase and chymase. Mast cells in turn produce and store cytokines. Murine mast cells have been shown to produce among other cytokines IL-1 beta, IL-6 and TNF alpha which is a potent preformed proinflammatory cytokine found also in human mast cells. The elegant experiments of Malviya et al. and Echtenacher et al. have shown a protective role for mast cells in acute bacterial peritonitis that is blocked by anti-TNF alpha antibodies. In Maleviya experiments W/Wv mast cells deficient mice were
20 fold less efficient that wild type or reconstituted mice in clearing enterobacteria when injected into the peritoneal cavity. TNF alpha from mouse mast cells was found essential for the clearance of Klebsiella Pneumoniae in a peritonitis model (44).

MIGRATION OF MAST CELL

Epithelial migration of mast cells has been suggested in vivo by the observations of Enerback et al. (45). Patients with allergic rhinitis before the pollen season were found to have mast cells in the connective tissue of the nasal mucosa with a redistribution occurring during the pollen season. An increased number of mast cells in the epithelium in close contact with the surface of the nose was seen but the total number of mast cells was not changed indicating an allergen induced mast cell migration. Data from mice infected with Trichinella Spiralis has confirmed that mast cells sequentially express different proteases as they progressively appear in the submucosa, lamina propria and epithelium mucosa at the height of the infection (46). In the recovery of the disease mast cell migrate to the villi and submucosa and alter their protease phenotype to accommodate to the tissue specific phenotype. It is clear that mast cell can reversibly alter their granule phenotype but no studies are available to identify which surface membrane structures are directly involved in the migration process and what soluble or cell bound ligands direct and orchestrate it.

1. ADHESION MOLECULES

Mast cell phenotypes are influenced by tissue factors that affect not only the expression of granule proteases and membrane mediators but also the expression of surface molecules. Surface and cell adhesion molecules that mediate mast cell attachment to extracellular matrix proteins (ECM) and to other tissue resident cells are important factors during mast cell differentiation, migration and homing (47).

In the connective tissue mast cells interact with the extracellular matrix proteins such as fibronectin, laminin and vitronectin and resident cells such as fibroblasts. The adherence of mast cells to fibroblasts is in part mediated by the interaction of fibroblast membrane-bound SCF with the c-kit receptor on mast cells (48). Mast cells adhere spontaneously to vitronectin (49), but need activation with PMA or FceRI crosslinking to adhere to fibronectin (50) and laminin (51). The adhesion to fibronectin is in part regulated by SCF, since it promotes adhesion of bone marrow-derived mast cells cultured in IL-3 to fibronectin in a dose-response fashion and at concentrations in the range of the plasma concentration of SCF (52). This interaction is RGD-peptide dependent, indicating that alpha 4 beta 1 and/or alpha 4 beta 7 integrins on mast cells are presumably the ligands for fibronectin. Expression of alpha 4, beta 1 and beta 7 genes has been shown in murine mast cells, and monoclonal antibodies have mapped the alpha 4, beta 1 and beta 7 proteins to the surface membrane (53). Engagement of matrix components and mast cell adhesion ligands can induce cell motility, intracellular proteins phosphorylation (49) and histamine and cytokine (TNF alpha) release (54). For example, FceRI-mediated activation of RBL-2H3 cells is enhanced by adhesion of cells to fibronectin-coated surfaces (55). The transcription of integrin genes is modulated during mast cell differentiation such that beta 1 and beta 7, but not alpha 4, transcripts are expressed in CTMC (56). The integrin complexes synthesized by mast cells are modulated by different cytokines. BMMC grown in SCF alone transcribe alpha 4, but not beta 7 integrins and BMMC grown in IL-3 induce beta 7 but abrogate alpha 4 transcription (57). A novel integrin chain, alpha M290, is expressed by mouse BMMC and is the human homolog of alpha E integrin which is known to pair with the beta 7 chain in intraepithelial lymphocytes (IEL) (58). The alpha e beta 7 pair was found to be an unexpected new ligand for E cadherin, which mediates homotypic adhesion, and may have a role in the homing process of IEL cells (59). Mouse alpha M290 integrin expression is inducible by TGF beta and IgE mediated crosslinking. Supernatants from activated mast cells expressing surface alpha M290 induce the paracrine expression of alpha M290 on other cells (60). This indicates that soluble mast cell products are capable of modifying the surface expression of integrins on other cells, influencing their cell-cell or cell-ECM interactions. Recent data has shown that metachromatic tryptase-positive mast cells derived from human fetal liver in the presence of rSCF adhere spontaneously to vitronectin through alpha V beta 3 surface integrins (61).

IMMUNOGLOBULIN SUPERFAMILY

ACTIVATORY RECEPTORS

FCERI AND FCGRIII

The activation of mast cell can be initiated by the aggregation of FceRI by IgE and antigen but aggregated IgG is also able to activate mast cells as well (62). The nature of the FcgR receptors present on the surface membrane of mouse mast cells has been described recently and their in vivo functions are now starting to be apparent. Three distinct low affinity IgG Receptors FcgRIIb1, FcgRIIb2, and
FcγRIII are expressed by mouse mast cells and found to be specific for IgG1, IgG2a and IgG2b with no cross reactivity with IgE. FcγRIII is composed of three chains, one alpha and two gamma, but the FcγRIib1/b2 are single chain molecules. FcεRI and FcγRIII have a similar alpha chain which contains 2 immunoglobulin C2 extracellular domains, the FcεRI has a beta chain lacking in the FcγRIII and both have two associated gamma chains with ITAM motifs (Immunoreceptor Tyrosine Based Activation Motifs) (Figure 3). The associated g chains function is to couple the receptors to signaling pathways (55). The ITAM motifs have a central tyrosine that becomes phosphorylated by src kinases when the receptors are clustered and propagate the downstream signals by recruitment of specific substrates leading to the release of membrane and granule mediators (63). The expression of FcεRI on mast cells seems to be partly regulated by IgE since IgE deficient mice have little expression of FcεRI as compared to normal littermates. The expression of FcεRI in mast cell deficient mice and normal littermates can be greatly enhanced by treatment with IgE (56). This upregulation is functionally significant since it increases substantially mediator and cytokine release (60% for serotonin and 6% for IL-6) upon IgE activation.

Mouse BMMC IL-3 express FcγRIIb1 and b2 but little FcγRIII, whereas serosal mature mast cells (SMC) taken in vivo from the peritoneal cavity express primarily FcγRIII and some FcγRIIb1 (55). Crosslinking the FcγRs induces secretory granule mediator (beta-hexosaminidase) and membrane generated arachidonic acid metabolites (LTC4 and PGD2) release from SMC at similar levels to those obtained when crosslinking the FcεRI (56). Transfection studies in rat basophil leukemia (RBL) cells expressing surface FcγRII and FcγRIII have shown that only the FcγRIII expressing transfectants release serotonin, LTC4 and TNF alpha (56). Furthermore, these responses are dependent on the cytoplasmic sequence of the g chain containing the ITAM (55). During mast cell ontogeny, both FcεRI and FcγRs are expressed prior to granulation (56). The functional relevance of the FcγRs on mast cells may be inferred from the in vivo anaphylactic response in IgE-deficient mice. Those mice produce no measurable IgE but normal levels of IgG and upon sensitization and antigen challenge present anaphylactic features with histological evidence of tissue mast cell degranulation and systemic histamine release (55). This is consistent with IgG and FcγR activation of mast cells and mediator release such as that seen with cultured mast cells. FcγRIII receptors on mast cells play a significant role in the cutaneous Arthus reaction since mast cell deficient mice W/Wv when reconstituted in the skin with mast cells lacking FcγRIII had an attenuated response to immune complex mediated injury (56).

INHIBITORY RECEPTORS

1. FcγRII

In vitro c-kit + committed mouse bone marrow derived mast cell progenitors lacking metachromatic granules express FcγRIIb1 and FcγRIIb2 (57). Those receptors have been found to have endocytic capabilities but recent data suggest an important role as inhibitory receptors when crosslinked to the FcεRI. When FcγRII receptors are transducted into RBL rat mast cells and crosslinked to the FcεRI, IgE induced serotonin release is inhibited (58). The inhibition is reversible upon disengagement of the FcγRII receptors and depends on an intact intracellular sequence common to FcγRIIb1 and b2 which contains a central motif, the ITIM (Immunoreceptor Tyrosine based Inhibitory Motif) (Figure 4). This motif is shared by other inhibitory receptors of the immune system including the KIRs (Killing Inhibitory Receptors) present on NK cells and CTLA-4 on T lymphocytes. The ITIM motifs become phosphorylated in the central tyrosine by specific src family kinases and recruit signaling molecules with inhibitory function (59). Physiological stimulus that could crosslink both FcεRI and FcγRII include antigen aggregated with IgG during immunotherapy treatment, in which patients raise high titers of IgG against immunizing antigens. FcεRI mast cell activation can be controlled by the low affinity FcγRII when both receptors are clustered by the same stimulus.

2. THE GP49 FAMILY

A new immunoglobulin subfamily that is preferentially expressed on mouse mast cells and mononuclear macrophages, the gp49 family (60), has been characterized which has novel regulatory functions for mast cells. Three of the members have been cloned -- gp49A, gp49B1 and gp49B2 -- and two genes isolated. The A gene codes for gp49A and the B gene for gp49B1 and gp49B2 by alternate splicing (60). gp49A and gp49B1 are integral type I membrane glycoproteins, and gp49B2 is a secretory form, identical to gp49B1 but lacking the transmembrane domain. The extracellular domain of gp49 glycoproteins contains two C2 domains as in ICAM-2. FcγRIII and FcεRI alpha chains (60). A newly recognized subfamily that includes gp49B1, the human myeloid receptor for IgA, the bovine myeloid Fc receptor for IgG2 and the KIRs expressed on NK cells and on T cell subsets has emerged based on the amino acid
The expression of IgE is controlled by switch recombination.

4. CD40 LIGAND (CD40L) AND IGE SYNTHESIS

The expression of IgE is controlled by switch recombination events leading to the translation of e heavy-chains and the differentiation and survival of IgE-committed B cells. The two signals required to direct recombination to the constant region of e heavy chains are the presence of cytokines such as IL-4 and IL-13 and the direct interaction between CD40 on B cells and CD40 ligand (CD40-L) (\(\sigma_9\)). Classically T cells have been described as the source of IL-4 and shown to stimulate B cells through CD40-L (\(\sigma_9\)). Purified human skin and lung mast cells, the basophilic cell line KU812 and human leukemic HMC-1 mast cell line express CD40L by indirect immunofluorescence which can be upregulated by PMA and ionomycin in HMC-1 cells (\(\sigma_6\)). HMC-1 and KU812 cells induce the synthesis of IgE from purified tonsilar B cells after culture in the presence of IL-4 for 12 days in similar amounts to that obtained by T cell help. Highly purified lung mast cells stimulated with SCF were also found to support IgE production by B cells (\(\sigma_8\)). This study indicates that mast cells at sites of allergic inflammation such as skin, lung and gut could potentially provide direct stimulation to B cells to induce and/or increase the local production of specific IgE. Whether activation of resident tissue mast cells is required for sufficient expression of the CD40-L and whether the same mast cells can provide IL-4 or IL-13 to support the switch recombination event has not been addressed.

SUMMARY

As shown in this review, the study of mast cells is complex and challenging. Human and mouse mast cell precursors travel in the blood stream that can be phenotypically recognized. Although hematopoietic in nature, mast cells become permanent residents once located in the tissues and have complex interactions with the environment. Autocrine and paracrine functions of mast cells have been described, and modulation of their phenotype and functions occurs through soluble factors as well as cell bound receptors and adhesion molecules. The discovery of at least two inhibitory pathways and receptors (FcgRII and gp49B1) strongly suggests that mast cell homeostasis is maintained through the balance between activatory and inhibitory stimuli. Therapeutic approaches may be designed that can take advantage of the naturally occurring pathways for mast cell inhibition.

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